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# Reproductive disadvantage in an Indian strain of malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae) on selections with deltamethrin/synergized deltamethrin

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**Abstract**: The knowledge of reproductive fitness in susceptible and resistant mosquitoes could be important in planning and implementing mosquito control programs. The laboratory studies were carried out to reveal the variations in the reproductive fitness of parent (AnS) and resistant (AnR) strains of malarial vector *Anopheles stephensi* originating from Delhi, India on selections with deltamethrin ($AnD_L$) or the combination of deltamethrin and PBO (1:5) ($AnD_P$) at the larval stage and selections with deltamethrin at the adult stage ($AnD_A$). The reproductive fitness was evaluated in terms of the fecundity, fertility, egg hatchability and the length of gonotrophic cycles. The AnR strains exhibited 60% - 73% reduced duration of the gonotrophic cycles when compared with AnS strain. There was a considerable decrease in the egg production, ranging from 14.5% to 37.9% in AnR strains as compared to the AnS strain. The maximum reduction was observed in $AnD_{L40}$ strain with the highest levels of deltamethrin resistance. The results indicate the possible positive correlation between the levels of deltamethrin resistance and the reproduction disadvantage. This correlation was further confirmed by 19.4% to 30.9% decrease in the hatchability of eggs of the $AnD_{L40}$ strain with respect to that of the parent strain. A worth mentioning observation of the reduced reproductive fitness in $RD_p$ strains suggests the efficacy of synergized deltamethrin selections in not only decreasing the deltamethrin resistance levels but also reducing the frequency of resistant individuals. The diminished reproductive fitness in adult-selected strains possessing negligible resistance to deltamethrin implicates the higher usefulness of deltamethrin as an adulticide in comparison to as a larvicide against *An. stephensi*. The results suggest that the reduced reproductive fitness of resistant genotypes in the population can eliminate heterozygotes and resistant homozygotes by implementing different resistance-management strategies against *An. stephensi*.

**Key words**: *Anopheles stephensi*; deltamethrin resistance; piperonyl butoxide; reproductive fitness; fecundity; egg hatchability; gonotrophic cycle; reproductive disadvantage

## INTRODUCTION

In India, *Anopheles stephensi* is one of the most common species responsible for urban malaria. According to WHO (2005), malaria affects more than 300 million people in a year throughout the world and is one of the main causes of mortality in infants and young children. In 1998, about 20 000 people and an estimated 577 000 disability-adjusted life years were lost due to malaria in India. The control failures of this mosquito are primarily due to the development of multiple insecticide resistance against various insecticides, posing serious threat to human beings (WHO, 1992). Indian strain of *An. stephensi* has been reported to be resistant to dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), malathion and deltamethrin (Raghavendra and Subbarao, 2002). The future of vector control mainly relies on the strategies for the management of existing insecticide resistance in them as the loss in terms of human lives because of mosquito-borne diseases is irrevocable (ICMR Bulletin, 2007).

The assessment of biological characteristics of resistant populations can be very important in the management of resistance (Campanhola *et al.*, 1991) as the resistance management strategies often rely on the assumption of reduced fitness in vector populations associated with resistance genes (Bonning and Hemingway, 1991; Raymond *et al.*, 2001). It has been observed that resistant and susceptible strains of insects frequently differ in fitness components (Arnaud *et al.*, 2002). In the absence of insecticide pressure, in general, the resistance genes tend to drift out of vector populations and the resistant strains exhibit a reduced reproductive fitness (Roush and Plapp, 1982; Kono, 1987; Argentine *et al.*, 1989; Li *et*

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*al.*, 2002; Kumar *et al.*, 2009), reducing the frequency of resistant individuals over time (Roush and McKenzie, 1987; Arnaud and Haubruge, 2002). A trade-off between fitness and insecticide resistance may result from the pleiotropic effects of genes involved in resistance (McKenzie and Batterham, 1994; Arnaud *et al.*, 2002). However, in certain cases, the resistant strains may have a fitness advantage with stable frequency (Arnaud *et al.*, 2002), the resistance may not lead to any loss of fitness (Okoye *et al.*, 2007) or the strains may differ in fitness for reasons independent of resistance (Roush and McKenzie, 1987).

The efficacy of deltamethrin as one of the most potent insecticides against the different life stages of *An. stephensi* has been well documented (Sahgal and Pillai, 1993; Kumar *et al.*, 2004). The deltamethrin-resistant population of an Indian strain of *An. stephensi* has been reported to exhibit reproductive disadvantage as compared to its susceptible counterparts (Priyalaksmi *et al.*, 1999), whereas Okoye *et al.* (2007) recorded increased reproductive fitness in a deltamethrin-resistant African strain of *An. funestus*. Very few studies have been undertaken to investigate the reproductive fitness of deltamethrin-resistant strains though the knowledge of variations in reproductive fitness of deltamethrin-resistant individuals could facilitate in planning and implementing the vector control programs. Keeping that in view preliminary laboratory studies were carried out on the field-collected and insecticide-selected Indian strains of *An. stephensi* to study the sub-lethal effects of deltamethrin and synergized deltamethrin resistance on their reproductive fitness. The investigations were based on the study of duration of gonotrophic cycles, fecundity and egg hatchability in the field-collected parent and deltamethrin-selected strains of *An. stephensi*.

# 1 MATERIALS AND METHODS

## 1.1 Mosquito population

The present investigations employ the larvae and adults of *An. stephensi* originated from field-collected engorged female adults from Delhi, India. The colony was maintained in an insectary without any insecticide exposure at 28 ± 1℃, 80% ± 5% RH and a photoperiod of 14L∶10D (Kumar *et al.*, 2002). Adults were supplied with soaked deseeded raisins. Periodic blood meals were provided to female mosquitoes for egg maturation by keeping restrained albino rats in the cages. The eggs were collected in an enamel bowl lined with Whatman filter paper and were allowed to hatch in enamel trays filled with de-chlorinated water. Larvae were fed upon a mixture of yeast powder and grinded dog biscuits. The pupae formed were collected in enamel bowls and transferred to the cloth cages for adult emergence.

## 1.2 Chemicals

Technical grade deltamethrin with a purity of 98.8% was obtained from Roussel Uclaf, India. The piperonyl butoxide (PBO), a synergist of deltamethrin used in the selection studies to reverse the deltamethrin resistance in the resistant strains, was also procured from Roussel Uclaf, India. Insecticide-impregnated papers of deltamethrin (0.025%) were procured from World Health Organization, Geneva. The papers were stored at 4℃ and were used for a maximum of 3 times.

## 1.3 Larval susceptibility and selection studies

The larval bioassay was performed on the early 4th instar larvae in accordance with the WHO method for mosquito larvae (WHO, 1981a). The larvae, in groups of 25, were treated for 24 h with 1 mL ethanolic solution of the insecticide in 249 mL of water taken in glass jars. Controls were exposed to ethanol alone, and control mortality if any, was corrected using Abbott's formula (Abbott, 1925). The data were subjected to regression analysis and the $LC_{50}$ and $LC_{90}$ values were calculated in each bioassay.

Selection studies were carried out by exposing several batches of 400 – 500 larvae for 24 h in glass jars containing 249 mL of water and 1 mL of the appropriate insecticide solution in ethanol. In each selection, the concentration of insecticide was adjusted to the $LC_{90}$ level. The survived larvae were reared to the adults. The larvae of the next generation were subjected to the susceptibility tests and selected with the same insecticide at $LC_{90}$ level. These selection studies were continued for successive generations and separate larval studies were performed.

## 1.4 Adult susceptibility and selection studies

The adult susceptibility studies were carried out by the WHO procedure using standard WHO kits (WHO, 1981b). Freshly blood-fed 3 d old female adults were exposed in batches to 0.025% deltamethrin-impregnated papers and mortalities were recorded. For selection studies, several batches of 20 – 25 fully blood-fed mated females were exposed to deltamethrin so as to induce 90% mortality in the adults. The surviving females were used for obtaining the next generation which was further exposed to the deltamethrin selection pressure.

### 1.5 Strains used in present studies

The various strains selected for present studies are as follows:

**1.5.1** AnS-line: AnS strain (parental strain): field-collected strain with $LC_{50}$ to deltamethrin = 0.001194 mg/mL.

**1.5.2** AnR-line: (1) $AnD_L$ strain: deltamethrin larval-selected strain:

(a) $AnD_{L20}$ strain: AnS strain selected with deltamethrin at larval stage for 20 generations. $LC_{50}$ to deltamethrin = 0.024592 mg/mL; resistance ratio = 20.6.

(b) $AnD_{L40}$ strain: $AnD_{L20}$ strain selected with deltamethrin till 40 generations at larval stage. $LC_{50}$ to deltamethrin = 0.071722 mg/mL; resistance ratio = 60.1.

(2) $AnD_P$ strain: deltamethrin + PBO (1:5) larval-selected strain:

(a) $AnD_{P20}$ strain: AnS strain selected with synergized deltamethrin at larval stage for 20 generations. $LC_{50}$ to deltamethrin = 0.016273 mg/mL; resistance ratio = 13.6.

(b) $AnD_{P40}$ strain: $AnD_{L24}$ strain subjected to selection pressure of synergized deltamethrin at larval stage till 40 generations. $LC_{50}$ to deltamethrin = 0.035367 mg/mL; resistance ratio = 28.9.

(3) $AnD_A$ strain: deltamethrin adult-selected strain:

(a) $AnD_{A20}$ strain: AnS strain exposed to deltamethrin at adult stage for 20 generations. $LT_{50}$ to deltamethrin = 11.0 min; resistance ratio = 3.8.

(b) $AnD_{A40}$ strain: $AnD_{A20}$ strain subjected to deltamethrin exposure at adult stage for next 20 generations. $LT_{50}$ to deltamethrin = 17.5 min; resistance ratio = 6.1.

### 1.6 Reproductive potential studies

Batches of freshly emerged 100 mosquitoes, 50 males and 50 females, of each strain were released in the separate cages and fed on soaked deseeded raisins. Female adults were provided with blood meal after three days of emergence which marked the beginning of first gonotrophic cycle. Unfed females and dead mosquitoes were removed from cages after blood meal and surviving blood-fed females were counted. Enamel bowls half-filled with dechlorinated water were kept in the cages to collect the eggs laid by females. After the completion of first round of oviposition, surviving females were counted again and provided with a second blood meal after 24 h of starvation. The period from the beginning of one blood meal to that of the next blood meal was recorded as one gonotrophic cycle (Kumar *et al.*, 2009). Numbers of blood-fed females were recorded again and allowed to lay eggs. The surviving females were provided with another blood meal after the completion of second round of oviposition and the observations were repeated. The duration of three consecutive cycles was accounted in all the strains of *An. stephensi*.

The fecundity and hatchability of the mosquitoes were recorded during three consecutive gonotrophic cycles to assess their reproductive fitness. Each day a fresh ovitrap was kept in each cage. The eggs laid were collected and counted every day after each blood meal using a dissecting microscope at the magnification of 40 × until no further eggs were laid for at least 48 h. The daily records were documented separately. For each gonotrophic cycle, fecundity rate per female was calculated and the fecundity of AnS strain was compared with the fecundity of AnR strains. The fecundity of each strain during three cycles was also compared.

The eggs collected during each gonotrophic cycle were submerged in a tray filled with dechlorinated water and allowed to hatch separately. The hatched larvae and the per cent hatch were calculated in each instance by taking a ratio of the number of hatched larvae to the number of eggs laid. The per cent hatch of AnS strain was compared with the per cent hatch of AnR strains. The total duration of oviposition in each gonotrophic cycle was also recorded.

### 1.8 Data statistics and analysis

The results obtained were analyzed using Student's *t*-test with statistical significance considered for $P \leqslant 0.05$ using the computerized SPSS 11.5 Programme.

## 2 RESULTS

### 2.1 Larval/adult susceptibility and selection studies

In the AnR-line, $AnD_{L40}$ strain had the highest levels of resistance (60.1-fold) followed by $AnD_{P40}$ strain (28.9-fold). The $AnD_A$ strains exhibited very low levels of resistance to deltamethrin (3.8 to 6.1-fold) in comparison to the larval-selected strains.

### 2.2 Duration of gonotrophic cycles

Our investigations on the reproductive potential of *An. stephensi* revealed that the AnR strains exhibited a significant reduction of 60% - 73% ($P < 0.05$; 0.001 to 0.004) in the duration of 3 consecutive gonotrophic cycles as compared to the AnS strain (Table 1). The $AnD_A$ strains with lowest resistance to deltamethrin exhibited the shortest duration of gonotrophic cycles as compared to other resistant strains, though the difference in the duration was not significant ($P > 0.05$). Nevertheless, the duration of each gonotrophic cycle was partially imposed by the blood-feeding regime as the successive blood meals were offered only after a 24 h starvation period.

**Table 1 Duration (in days) of the three consecutive gonotrophic cycles of parent and insecticide-selected strains of *Anopheles stephensi***

| Strain | 1st gonotrophic cycle | 2nd gonotrophic cycle | 3rd gonotrophic cycle | Total duration ** |
|---|---|---|---|---|
| AnS | 9.0 ± 0 * | 9.0 ± 0 | 7.0 ± 0 | 25.0 a |
| AnD$_{L20}$ | 7.0 ± 0 | 6.0 ± 0 | 5.33 ± 0.33 | 18.33 b |
| AnD$_{L40}$ | 7.0 ± 0 | 5.0 ± 0 | 4.33 ± 0.33 | 16.33 b |
| AnD$_{P20}$ | 6.0 ± 0 | 6.0 ± 0 | 5.33 ± 0.33 | 17.33 b |
| AnD$_{P40}$ | 6.0 ± 0 | 6.0 ± 0.33 | 4.66 ± 0.33 | 16.66 b |
| AnD$_{A20}$ | 6.0 ± 0 | 5.0 ± 0 | 4.0 ± 0 | 15.0 b |
| AnD$_{A40}$ | 6.0 ± 0 | 5.0 ± 0 | 5.0 ± 0 | 16.0 b |

* Mean ± *SE* of 3 replicates. ** Figures in the column followed by different letters are significantly different at $P = 0.05$ (Student's *t*-test).

## 2.3 Reproductive potential studies

**2.3.1** First gonotrophic cycle: The present studies clearly demonstrated significant reduction in fecundity of AnD$_L$ strains during the 1st gonotrophic cycle (Table 2). The most drastic and significant effect was observed in the AnD$_{L40}$ strains with 14.5% ($P = 0.02$) and AnD$_{L20}$ strain with 11.6% reduced fecundity ($P = 0.04$). This suggests that 2.9-fold increased levels of deltamethrin resistance (from 20.6-fold to 60.1-fold) resulted in 3% decreased fecundity in the AnD$_L$ strains of *An. stephensi*. In synergized deltamethrin and adult-selected AnR strains, the decrease in fecundity ranging from 5.4% – 10% (Table 2) was not significant ($P > 0.05$). These results indicate that resistance to deltamethrin alone developed at the larval stage resulted in more reproductive disadvantage as compared to that developed at adult stage or developed after larval selections with synergized deltamethrin.

**Table 2 Effect of deltamethrin and synergized deltamethrin selections on the fecundity and hatchability of *Anopheles stephensi* in the 1st gonotrophic cycle**

| Strain | Number of blood-fed females | Days of oviposition | Total number of eggs laid | Number of eggs laid per female # | Relative fecundity * | Total hatch | Hatch per female # | % Hatch ** |
|---|---|---|---|---|---|---|---|---|
| AnS | 35 | 5 | 3 780 | 108.00 a | – | 3 416 | 97.6 a | 90.4 |
| AnD$_{L20}$ | 35 | 4 | 3 374 | 96.40 b | 89.2 | 2 534 | 72.4 bc | 75.1 |
| AnD$_{L40}$ | 35 | 3 | 3 234 | 92.40 b | 85.5 | 2 296 | 65.6 c | 71.0 |
| AnD$_{P20}$ | 35 | 3 | 3 591 | 102.60 a | 95.0 | 2 814 | 80.4 b | 78.4 |
| AnD$_{P40}$ | 35 | 3 | 3 535 | 101.00 a | 93.5 | 2 786 | 79.6 b | 78.8 |
| AnD$_{A20}$ | 40 | 2 | 4 000 | 100.00 a | 92.6 | 3 136 | 78.4 b | 78.4 |
| AnD$_{A40}$ | 35 | 3 | 3 430 | 98.00 ab | 90.7 | 2 639 | 75.4 bc | 76.9 |

* Fecundity calculated as percentage of the number of eggs laid per female of the AnS strain. ** Calculated by dividing total hatch from the total number of eggs laid. # Figures in each column followed by different letters are significantly different at $P = 0.05$ (Student's *t*-test). The same for Tables 3 and 4.

The studies on the hatchability during the 1st gonotrophic cycle confirmed the fact that the females of all the AnR strains were significantly less fertile (65 – 80 hatched eggs per female) in comparison to the parent strain with 98 hatched eggs ($P < 0.05$) (Table 2). The eggs of AnD$_{L40}$ and AnD$_{L20}$ strains showed only 71% – 75% hatching as against 90% hatching in AnS strain ($P = 0.008$; $P = 0.01$) exhibiting 15% – 19% reduced egg hatchability while other strains showed 11% – 13% decreased hatchability in contrast to the parent strain.

**2.3.2** Second gonotrophic cycle: The second gonotrophic cycle displayed further reduced fecundity rates in all strains of *An. stephensi* as against that of in the 1st cycle (Table 3). The results followed the same pattern as in the 1st cycle with AnD$_{L40}$ and AnD$_{L20}$ strains exhibiting the maximum and significant reproductive disadvantage of 36% – 39% ($P = 0.001$). This again suggested a negative relationship between the deltamethrin resistance at larval stage and the reproductive fitness. It was interesting to note that in contrast to the first cycle, the adult-selected AnD$_{A20}$ strain exhibited the increased relative fecundity from 93% to 98%.

The hatchability rates of 2nd gonotrophic cycle revealed the AnD$_{L40}$ strain to be the significantly least fertile with only 31.5 larval hatch per female and 30% reduced hatchability in relation to the AnS strain. The other selected strains also showed an appreciable reduction in the per cent hatch displaying 12% to 27% lower hatchability potential ($P < 0.05$) (Table 3) except AnD$_{A20}$ strain (3.8-fold resistant to deltamethrin) which showed only 6% reduced fertility. This further stresses upon our finding that reproductive

**Table 3　Effect of deltamethrin and synergized deltamethrin selections on the fecundity and hatchability of *Anopheles stephensi* in the 2nd gonotrophic cycle**

| Strain | Number of blood-fed females | Days of oviposition | Total number of eggs laid | Number of eggs laid per female$^{\#}$ | Relative fecundity$^{*}$ | Total hatch | Hatch per female$^{\#}$ | % Hatch$^{**}$ |
|---|---|---|---|---|---|---|---|---|
| AnS | 32 | 5 | 2 752 | 86.00 a | – | 2 408 | 75.2 a | 87.5 |
| AnD$_{L20}$ | 32 | 3 | 1 944 | 60.75 c | 70.6 | 1 376 | 43.0 c | 70.8 |
| AnD$_{L40}$ | 32 | 2 | 1 752 | 54.75 cd | 63.7 | 1 008 | 31.5 d | 57.5 |
| AnD$_{P20}$ | 28 | 3 | 1 974 | 70.50 b | 82.0 | 1 351 | 48.2 bc | 68.4 |
| AnD$_{P40}$ | 28 | 3 | 1 876 | 67.00 bc | 77.9 | 1 134 | 40.5 c | 60.4 |
| AnD$_{A20}$ | 32 | 2 | 2 688 | 84.00 a | 97.7 | 2 192 | 68.5 a | 81.5 |
| AnD$_{A40}$ | 28 | 2 | 2 163 | 5.73 b | 88.0 | 1 638 | 58.5 b | 75.7 |

fitness of *An. stephensi* has negative correlation with the deltamethrin resistance levels. Again, the AnS strain had longer oviposition period than the selected strains, though significantly not different ($P > 0.05$).

**2.3.3** Third gonotrophic cycle: Further reduction in the egg laying capacity was noticed in the 3rd gonotrophic cycle in all the strains of *An. stephensi* with pattern similar to the earlier cycles. The maximum reduced fecundity was observed in AnD$_{L40}$ strain with 27% reduced fecundity as compared to AnS strain and 21% as compared to the 2nd cycle (Table 4). A remarkable observation was the diminished egg production in AnD$_{P40}$ strain which laid 26% less number of eggs in the 3rd cycle. A reduced fecundity of 8% – 15% was noticed in the adult-selected strains.

**Table 4　Effect of deltamethrin and synergized deltamethrin selections on the fecundity and hatchability of *Anopheles stephensi* in the 3rd gonotrophic cycle**

| Strain | Number of blood-fed females | Days of oviposition | Total number of eggs laid | Number of eggs laid per female$^{\#}$ | Relative fecundity$^{*}$ | Total hatch | Hatch per female$^{\#}$ | % Hatch$^{**}$ |
|---|---|---|---|---|---|---|---|---|
| AnS | 28 | 3 | 1 960 | 70.00 a | – | 1 722 | 61.5 a | 87.8 |
| AnD$_{L20}$ | 24 | 2 | 1 230 | 51.50 b | 73.8 | 804 | 33.5 c | 65.4 |
| AnD$_{L40}$ | 28 | 1 | 1 218 | 43.50 c | 62.1 | 693 | 24.8 d | 56.9 |
| AnD$_{P20}$ | 24 | 2 | 1 272 | 53.00 b | 75.7 | 912 | 38.0 bc | 71.7 |
| AnD$_{P40}$ | 20 | 1 | 990 | 49.50 bc | 70.7 | 630 | 31.5 cd | 63.6 |
| AnD$_{A20}$ | 28 | 1 | 1 736 | 62.00 b | 88.6 | 1 225 | 43.8 b | 70.6 |
| AnD$_{A40}$ | 24 | 2 | 1 326 | 55.25 b | 78.9 | 822 | 34.2 c | 62.0 |

Similar trend was recorded in the hatchability rates of various strains with the percent hatch decreasing to an appreciable level in AnD$_{A20}$ and AnD$_{A40}$ strains in comparison to that in the earlier cycles. The AnD$_{L40}$ strain proved to be the least fertile (57%) among all the AnR strains studied. The noticeable observation was the 3% increased fertility in the AnD$_{P}$ strains as compared to the previous cycle (Table 4). In addition, no marked difference in the oviposition durations was seen in the 3rd cycle ($P > 0.05$).

## 3 DISCUSSION

### 3.1 Larval/adult susceptibility and selection studies

The high insecticidal potential of deltamethrin against various species of mosquitoes is well established (Kumar *et al.*, 2002, 2004). The present studies also confirmed the efficacy of deltamethrin against field-collected strain of *An. stephensi*, though the successive selections of parent susceptible strain with deltamethrin alone caused the development of an appreciable level of resistance to deltamethrin. The studies also revealed that the use of synergized deltamethrin along with PBO though reduced the levels and speed of deltamethrin resistance yet could not block it completely. The investigations on reproductive fitness of the susceptible and variously-selected resistant strains reveal the fact that the deltamethrin-resistant population of *An. stephensi* showed variability in the reproductive potential; in general with an appreciable reproductive disadvantage. The studies suggest that increased levels of resistance to deltamethrin reduce the reproductive fitness of *An. stephensi*. Resistance management tactics may rely on the reduced fitness of resistant genotypes relative to susceptible genotypes with the aim of preserving susceptible homozygotes and eliminating heterozygotes and resistant homozygotes (Leeper *et al.*, 1986). The resultant reproductive disadvantage is thus of high significance in vector control as it would probably decrease the resistant individuals in the fields over generations.

### 3.2 Duration of gonotrophic cycles

Our investigations revealed the duration of the successive gonotrophic cycles to be higher in the AnS strain compared to the AnR strains of *An. stephensi*. Our results differ from the results of Priyalakshmi *et al.* (1999) who recorded longer life cycle in the Indian strain of deltamethrin-treated *An. stephensi* as compared to the non-treated control strain, but are similar to those reported in an Indian strain of deltamethrin-resistant *Ae. aegypti*, obtained after selection with deltamethrin for forty successive generations (Kumar *et al.*, 2009).

### 3.3 Reproductive potential studies

The studies also proved that deltamethrin-resistant $\mathrm{AnD_{L40}}$ (60.1-fold resistance) and $\mathrm{AnD_{P40}}$ (28.9-fold resistance) strains of *An. stephensi* possess much lower fecundity as compared to the $\mathrm{AnD_A}$ and AnS strains. These results clearly indicate that the larval-selected strains with appreciable levels of deltamethrin exhibited much more reproductive disadvantage as compared to those with low levels of resistance. One probable reason for the reduced fecundity in the selected strains as compared to the parent strains may be the penetration of insecticides into the ova imparting toxic effects on the egg follicle growth which possibly caused the inhibition of synthesis and transport of egg yolk proteins. It indicates a negative correlation between the levels of deltamethrin larval resistance and the reproductive potential of *An. stephensi*. Similar results have been reported by Priyalakshmi *et al.* (1999) in an Indian strain of *An. stephensi* which exhibited 32% – 40% decrease in fecundity rates when treated with sublethal doses of deltamethrin at the larval stage. Mohapatra *et al.* (1999) had also reported significant decrease in the fecundity rates of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* after treatments with cyfluthrin and fenfluthrin. Earlier, Verma (1986) also recorded reduced fecundity of 40.6%, 43.3% and 67.2% after selection pressures of pyrethroids in *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Our results are comparable with those reported in an Indian strain of *Ae. aegypti* which exhibited 36.7% reduced fecundity when selected with deltamethrin for 40 generations at larval stage (Kumar *et al.*, 2009). In a Chinese strain of *Cx. pipiens pallens* with 617-fold deltamethrin resistance, Li *et al.* (2002) reported 44.8% reduced fecundity after 12 generations of deltamethrin selection.

The interesting observation in our investigations was that the $\mathrm{AnD_{A20}}$ strain with almost negligible levels of deltamethrin resistance did not show any remarkable reduction in the fecundity, which again proved that there may be a correlation between the reproductive fitness and the levels of deltamethrin resistance. Further, the reduced fecundity of $\mathrm{AnD_{A20}}$ in the 1st gonotrophic cycle was regained in the 2nd cycle, though it exhibited reduced fecundity again in third cycle. The decrease and then regain of the reduced fecundity in successive gonotrophic cycles remained unclear and needs further explorations.

Our studies also reveal that in the successive gonotrophic cycles, the parental population exhibited maximum hatch in contrast to the insecticide-selected strains; further reducing gradually in later cycles. The $\mathrm{AnD_{L40}}$ strain proved to be least fertile in three consecutive cycles exhibiting lowest per cent hatch of 57% to 71%. Priyalakshmi *et al.* (1999) had revealed 24% decrease in the fertility rates of an Indian strain of *An. stephensi* when treated with sublethal doses of deltamethrin at larval stage. On the contrary, in an African strain of *An. funestus*, Okoye *et al.* (2007) observed 81.5% larval hatch in the pyrethroid-resistant strain as compared to 66.9% hatch recorded in the susceptible strain. In an Indian strain of *Ae. aegypti*, Kumar *et al.* (2009) reported the least fertility in $\mathrm{F_{40}}$ deltamethrin-larval selected strain for the first two gonotrophic cycles, whereas in third cycle, the least fertile strain reported was $\mathrm{F_{40}}$ synergized deltamethrin larval-selected strain. Significant reduction in the fertility rates of Indian strains of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* was recorded after exposure to cyfluthrin and fenfluthrin (Mohapatra *et al.*, 1999).

The lowest fertility in $\mathrm{AnD_{L40}}$ strain with highest level of larval deltamethrin resistance (60.1-fold) again indicates a negative impact of the insecticide resistance on the hatchability rate of *An. stephensi*. The possible cause may be the selection pressure of insecticides at higher dosage as compared to the dosage subjected to the less-resistant strains as deltamethrin has been reported to have delayed ovicidal effects in *An. stephensi* (Sahgal and Pillai, 1993) leading to the penetration of insecticides in the ova and reduced egg hatchability.

Our studies show that the $AnD_A$ strains of *An. stephensi* developed very low levels of resistance to deltamethrin even after successive generations of deltamethrin selection at the adult stage, but these strains showed reduced fecundity and fertility, though not as much as in the case of the $AnD_L$ strains. It proves that despite the fact that the development of deltamethrin resistance in *An. stephensi* is stage-specific, the reduction in reproductive fitness is irrespective of its stage. This is an important and valuable observation as the efficacy of deltamethrin as an adulticide, failure of adult selection to induce the development of deltamethrin resistance in the adults and the ability of deltamethrin selections to cause reproductive disadvantage in the surviving adults suggest the long-term and efficient use of deltamethrin against malarial vector.

The larval selection of *An. stephensi* with synergized deltamethrin in the present studies confirmed the low reproductive fitness in $AnD_P$ strains. It suggests that the selections with synergized deltamethrin not only retards or reverses the deltamethrin resistance but it also provides reproductive disadvantage to the strain and reduces its reproductive fitness. This may help in reducing the frequency of resistant individuals and could be useful in planning and implementing strategies for mosquito control.

## 3.4 Conclusion

Our investigations emphasize the fact that deltamethrin is an effective insecticide against the control of malarial mosquito. Continuous subjection of selection pressure in *An. stephensi* caused the development of significant levels of larval resistance to deltamethrin which could be reduced after selection with synergized deltamethrin. Despite the development of resistance in various strains, deltamethrin holds great promise as a control agent because of the negative correlation of deltamethrin resistance levels with reproductive fitness. The lowest fecundity and fertility in $AnD_{L40}$ strain with highest level of larval deltamethrin resistance and thereby increased reproductive potential in $AnD_P$ proved the correlation. Further, the efficacy of deltamethrin as an adulticide and ability to reduce reproductive potential of adult mosquitoes, in $AnD_A$ strains, to an appreciable level suggest that the use of deltamethrin as an adulticide rather than as a larvicide against *An. stephensi* is a more effective strategy from a resistance-management perspective. The results clearly suggest that the reduced reproductive fitness of resistant genotypes in the population can eliminate heterozygotes and resistant homozygotes by implementing different resistance-management strategies against *An. stephensi*.

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# 疟疾媒介斯氏按蚊印度品系经溴氰菊酯或溴氰菊酯增效剂选择后的繁殖劣势

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**摘要**：了解敏感和抗性蚊虫的繁殖适合度对于规划和实施蚊虫防治计划具有重要意义。本研究分别将斯氏按蚊 *Anopheles stephensi* 幼虫用溴氰菊酯（$AnD_L$）及溴氰菊酯和 PBO 混配制剂（1∶5）（$AnD_P$），或斯氏按蚊成虫用溴氰菊酯（$AnD_A$）进行选择后，在实验室内检测了起源于印度德里的斯氏按蚊亲本（AnS）和抗性品系（AnR）的繁殖适合度的变化，从繁殖力、生育力、卵孵化率和生殖营养周期的长度等方面评价了斯氏按蚊的繁殖适合度。结果表明：与 AnS 品系相比，AnR 品系的生殖营养周期缩短了 60% ~73%。与 AnS 品系相比，AnR 品系的产卵量显著降低，降幅达 14.5% ~37.9%，对溴氰菊酯抗性最强的 $AnD_{L40}$ 品系的产卵量降低得最多。这些结果说明溴氰菊酯抗性与繁殖劣势之间可能存在正相关。与亲本品系相比，$AnD_{L40}$ 品系的卵孵化率降低了 19.4% ~30.9%，进一步证实了这一相关性。在 $RD_P$ 品系中观察到繁殖适合度降低，表明溴氰菊酯增效剂的选择不仅在降低溴氰菊酯抗性水平而且在降低抗性个体频率上的效率。在对溴氰菊酯几乎不具有抗性的成虫品系的选择中繁殖适合度降低，暗示溴氰菊酯作为灭杀斯氏按蚊成虫剂的效果要好于作为杀幼虫剂的效果。这些结果提示，通过对斯氏按蚊实施不同抗性治理策略，种群中抗性基因型的繁殖适合度的降低可消除杂合子和抗性纯合子。

**关键词**：斯氏按蚊；溴氰菊酯抗性；增效醚；繁殖适合度；繁殖力；卵孵化率；生殖营养周期；繁殖劣势

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